Title: Prediction of Varicocelectomy Outcomes Using 1 H NMR-based Metabonomics Assays of Blood Serum

Objectives: To create metabolomics models capable of segregating men who improved semen analysis (SA) parameters after microsurgical varicocelectomy (MV) from those who did not, using Hydrogen-1 nuclear magnetic resonance (1H NMR) spectra of blood serum from of pre-operative samples.

Methods: We recruited 32 infertile men with palpable varicocele and abnormal semen analysis (SA). Preoperative peripheral venous blood samples were centrifuged at 3000 g for 15 minutes to separate the cellular component from the supernatant serum. The blood serum samples were kept frozen at –40°C until analysis. 1H NMR spectra of blood serum were obtained and used to create metabonomics models. All participants were treated with artery and lymphatic sparing MV by the same surgeon. Improvement was defined as an increase of at least 20% in the total motile progressive sperm count (TMC) of the post-operative SA when compared to the baseline.

Results: Eighteen (56%) participants had SA improvement after MV and were included in the improved (I) group, the remaining 14 participants were included in the non-improved (NI) grop. There were no differences regarding the baseline characteristics and SA parameters. The I group had lower baseline luteinizing hormone levels when compared to the NI group (4.3 vs 7.0 mUI/mL), and there were no other differences concerning the baseline sexual hormones levels. The I group showed higher post-operative sperm concentration, total sperm count, progressive motility, TMC, and morphology than the group non-improvement group (Table 1). Concerning the changes of TMC from baseline, the I group had a median increase of 10.6 x 106 sperm whereas the group NI had a median decrease of -5.1 x 106 sperm (P-value < 0.05). When the changes were analyzed using percentage, this difference was further highlighted, the group I demonstrated an increase of 223% (range 44% to 34000%) and the group NI had a decrease of -59% (range -9% to – 100%) (P-value < 0.05).Using Linear Discriminant Analysis (LDA), we created a model that discriminated the men who improved SA from those who did not with sensitivity of 88.2%, specificity of 80.0% and accuracy of 84.4% after leave-one-out cross validation. We identified 4 metabolites that were important for group segregation: low density lipoprotein, isoleucine, glutamate, and methylamine.

Conclusions: We described the use of metabonomics model to predict the outcomes of MV in infertile men with varicocele with high accuracy. The most important metabolites for groups segregation are involved in amino acid metabolism and oxidative stress response, highlighting the pivotal role of these mechanisms in the pathophysiology of varicocele. These models may help counseling infertile men with varicocele regarding their prognosis after surgery.

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| Parameter | **Non-improved (n=14)** | **Improved (n=18)** | **P value** |
| Time since surgery (days), median (IQR) | 120 (100, 150) | 105 (90, 150) | 0.36 |
| Sperm concentration (106/mL), median (IQR) | **0.6 (0.0, 7.5)** | **17.0 (4.5, 23.5)** | **<0.05** |
| Total sperm count (106/mL), median (IQR) | **2.9 (0.0, 30.8)** | **41.0 (13.8, 63.5)** | **<0.05** |
| Progressive motility (%), median (IQR) | **20 (0, 32)** | **37 (19, 45)** | **<0.05** |
| Total progessive motile sperm count (106/mL), median (IQR) | **0.1 (0.0, 7.3)** | **14.2 (4.9, 29.6)** | **<0.05** |
| Morphology (%), median (IQR) | **5.0 (2.5, 8.5)** | **8.0 (5.0, 10.0)** | **<0.05** |
| Volume (mL), median (IQR) | 3.5 (1.9, 5.0) | 2.5 (2.0, 4.0) | 0.69 |
| pH, median (IQR) | 8.0 (7.0, 8.0) | 7.8 (7.3, 8.0) | NA |
| Change TMC (106/mL), median (IQR) | **-5.1 (-10.0, -1.0)** | **+10.6 (+2.6, +25.3)** | **<0.05** |
| Change TMC (%), median (IQR) | **-59 (-94, -34)** | **+223 (+94, +1500)** | **<0.05** |